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Synchronous Mass Release of Mature Medusae from the Hydroid *Halocordyle disticha* (Hydrozoa, Halocordylidae) and Experimental Induction of Different Timing by Light Changes

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Abstract The timing mechanism for synchronous mass release of mature medusae of *Halocordyle disticha* was studied, using colonies from Shirahama, Wakayama, Japan, which were kept in a 450 l aquarium tank. In near natural conditions medusa release is correlated with sudden drop of light intensity such as occurs around sunset. Timing could be manipulated by controlling light intensity. Artificial sunset 2 hours earlier than normal caused mass release of medusae earlier than under natural conditions, whereas sunset artificially delayed 3 hours later than normal caused continuous release of medusa after the onset of darkness. The spawning of gametes of *H. disticha* is almost simultaneous with medusa release, and since the medusa has an ephemeral planktonic existence, synchrony of mass medusa release and also spawning of gametes may maximize fertilization success.

Key words: hydroids, medusa release, release of gametes, timing, light change, *Halocordyle disticha*

Introduction

Marine organisms often reproduce circa-tidally, circa-semilunarly, or with some circadian periodicity. In the Cnidaria, simultaneous spawning by more than 100 species of scleractinian corals on the Great Barrier Reef in Australia during the spring full moon is well known (Babcok *et al.*, 1986; Fautin *et al.* 1989). In hydrozoans, synchronous spawning of gametes at a particular time of day occurs in both the medusae stage and polyp stage with gonophores (Ballard, 1942; Roosen-Runge, 1962; Miller, 1979, Honegger *et al.*, 1980; Yoshida *et al.*, 1980), but the precise timing of medusa release has not been widely reported.

Different environmental factors may trigger medusa release. Medusa development in some hydrozoans occurs only in seasons when conditions are optimal, and it may be suppressed at other times (Cornelius, 1990). Medusa release in *Obelia* has been linked to the phase of the moon (Elmhirst, 1925), and circadian periodicity in medusoid release has been found in some species of several genera of hydroids (Teissier, 1922; Brinckmann-Voss, 1970). Kubota (1996) detected a constant pattern of medusa release in *Eugymnanthea japonica* Kubota, 1979, a hydrozoan commensal of bivalves. This species releases medusae for a few hours a day around sunset in the warm season. Kubota (1997) clarified the differences in time of release between *Eugymnanthea japonica* and *Eutima japonica* Uchida, 1925, another bivalve-inhabiting species distributed in Japan. Synchronized release of medusae has also been reported from three species of hydrozoan fire corals by Soong and Cho (1998).

In the present study we analyse the release of medusae of *Halocordyle disticha* (Goldfuss, 1820) (Anthomedusae, Halocordylidae). Because that medusae have a short planktonic life, their simultaneous release would be advantageous for reproductive success. We thus tried to confirm the existence of a timing mechanism for the release of the medusae. Factors that may act as cues for simultaneous release were screened by controlling the culture conditions.

Material and Methods

Halocordyle disticha is a species with a circumglobal distribution in tropical and subtropical waters (Mammen, 1963; Millard, 1975; Hirohito, 1979). This hydrozoan is one of a few Anthomedusae that have an upright stem with a regular pattern of branching; other arrangements have been described, though such as bushy to pinnate forms that have been considered morphological variations due to different environmental conditions (Silveira and Migotto, 1991). In any colony form, the hydranths have an aboral whorl of filiform tentacles and an oral set of short, capitate tentacles amid which medusa buds are produced. The gonophores are eumedusoids with four rudimentary marginal warts, four radial canals, and a long manubrium with a closed mouth. Extensive discussions of the synonymy of *H. disticha* were given by Hirohito (1979) and Garcia-Corrales and Aguirre (1985).

Colonies of *H. disticha* were collected from sublittoral rocky substrata at Shirahama, Japan (33°41′N - 135°20′E) between July 4 and August 27, 1999. Pieces of rocks on which hydroids were growing were removed by means of a hammer and placed in plastic bags, then immediately carried back to the aquarium room. Macrobenthic organisms such as polychaetes, crustaceans, and molluscs were cleaned off, then the hydroids were put into vertically suspended plankton net (mesh-size 0.34 mm) with the mouth held 5 - 10 cm out of the water. The net was 30 cm across the mouth and 70 cm in side length, ending in a cylindrical plastic tube, 37 mm in diameter, with a short rubber pipe attached as a pinchcock in place of a stopcock. The nets were put into an aquarium tank of 450 l volume with aerated and constantly running sea water. The tank was on the top of floor of the Aquarium building, where natural sunlight was available.

The initial state of each colony was measured, including the hydrocaulus number, colony height, total number of branches, and number of zooids (Table 1). During the period of this study, the water temperature was measured several times daily. Light intensity was also measured using a photocell illuminometer (TOPCON SPI - 71) several times each day.

Colonies were fed freely with *Artemia* nauplii at the same time (10:00 a.m.) every day. Collection of medusae was initiated 5 or more hours after field collection. Medusae were collected up to 10 times per day (minimum interval, 15 minutes) and numbers were counted immediately under a stereomicroscope. At the same time, swimming activity of the medusae was checked and scored as either (S: swimming, P: not swimming but pulsating on the bottom of the petri dish, or N: neither swimming nor pulsating, indicating no movement of medusae even when they were prodded with a

Table 1. Morphological characteristics of *Halocordyle disticha* colonies, used for experimental induction of medusa release.

Colony N°	Coll. date (1999)	No. of hydrocauli	Max. height (cm)	No. of branches	No. of zooids	Sex	Exper. cond.*
1	July 3	9	11	202	670	male	Nc
2	July 11	7	11	147	514	female	Nc
3	July 11	8	8	144	504	male	Nc
4	July 14	12	9	156	500	female	Nc
5	July 19	6	7	78	280	female	Nc
6	July 25	3	10	40	120	female	Ads
7	July 25	11	13	210	590	female	Ads
8	July 29	15	12	270	720	male	Ads
9	Aug. 9	4	9	95	396	female	Ad
10	Aug. 12	3	10	45	135	female	Ad
11	Aug. 16	6	11	50	170	male	Ads
12	Aug. 25	5	8	85	260	female	Ad

* Nc: natural conditions. Ad: artificial darkness. Ads: artificially delayed sunset.
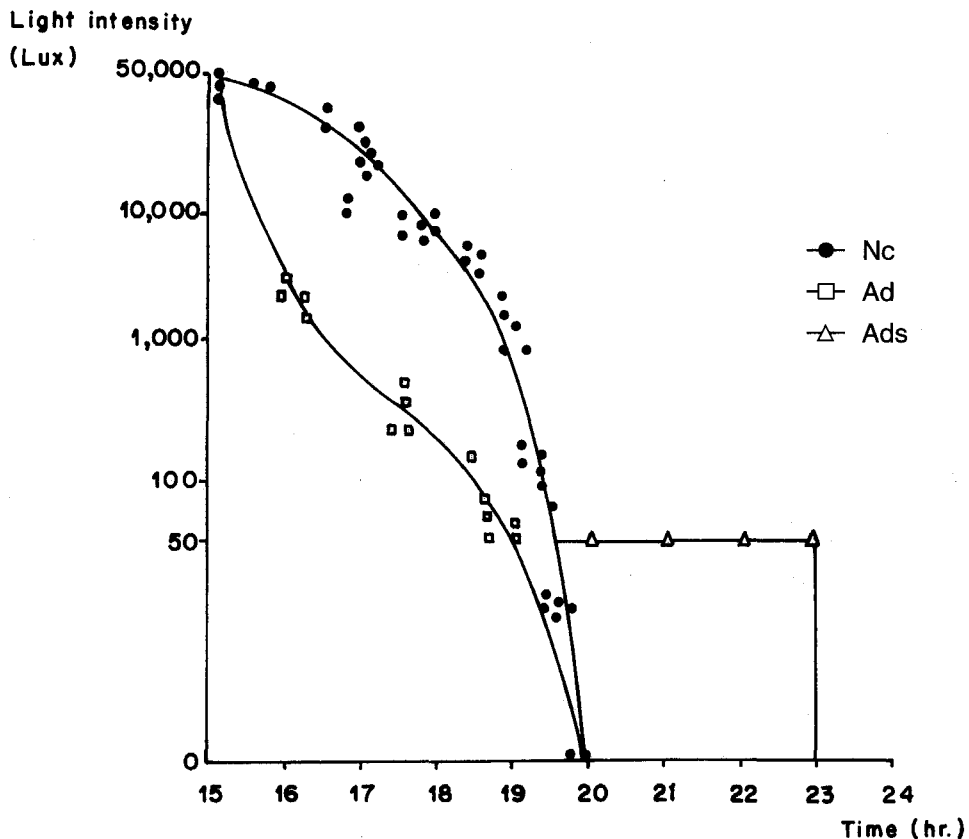
Fig. 1   Changes in light intensity (lux; logarithmic scale) between 15:00 and 24:00 hr (Nc: natural conditions; Ad: artificial darkness; Ads: artificially delayed sunset).

swimming nor pulsating, indicating no movement of medusae even when they were prodded with a needle).

Light conditions were manipulated in two ways. Artificial periods of darkness were introduced by covering the tank, thus simulating the onset of sunset two hours earlier than under natural conditions (Fig. 1). Artificially delayed sunset was produced by illuminating the tanks with room light for 3 hours past natural sunset.

Differences in the proportions of released medusae under different conditions were analyzed by mean of a chi-square test. Differences in sizes of male and female medusae was analyzed using an average difference test.

Results and Discussion

(1) Timing of the medusae release

Many collections of released medusae were made at different times each day, including sunrise and both low and high tide, but a total of 3710 medusae was found between 17:30 and 23:00 hr. Most of them were released between 18:45 and 20:00 hr (82.5%, N= 3062). Percentages of medusae released between 17:30 and 18:30 hr averaged 2.8% (N= 105) with a range of 0 - 5.5%. A more variable percentage (0-35.5%) was released between 20:15 and 23:00 hr (N= 543, mean 14.6%). The

Table 2. Temporal changes in the number of medusae released from hydroids of *Halocordyle disticha* (sunset was at 19:10 hr. in every day).

Date	Colony No.	17:00	17:15	17:30	17:45	18:00	18:15	18:30	18:45	19:00	19:15	19:30	19:45	20:00	20:15	20:30	20:45	21:00	21:15	21:30	21:45	22:00	22:15	22:30	22:45	23:00	Nc	Mc	Moon phase	Weather	T (°C)
July 4	1	...	...	...	...	...	...	0	...	...	...	...	...	23	...	...	...	...	...	...	4 *	...	...	...	...	3	4	30		c	24.6-25.0
July 5	1	...	...	...	...	0	...	...	...	6	...	...	...	2	...	...	...	...	...	...	...	...	...	*	...	...	3	8	lq	s	25.5-25.5
July 6	1	**	...	0	...	...	...	13	...	...	...	15	...	12	...	...	...	4	...	...	...	1	...	...	...	...	6	45		s	24.8-25.5
July 7	1	...	...	6	...	**	...	18	...	30	...	...	...	10	...	...	...	5	...	...	...	1	...	...	...	...	6	70		s	25.5-25.9
July 8	1	...	...	2	...	...	...	...	...	8	**	...	...	...	...	0	...	...	...	...	...	...	...	...	...	...	3	10		s	25.0-25.8
July 11	2	0	*	0	...	2	...	12	...	190	...	212	...	208	...	49	...	...	...	19	...	...	...	4	**	...	10	696		s	25.8-25.9
	3	0	...	0	...	0	...	10	...	25	...	28	...	55	...	43	...	...	...	17	...	...	...	5	...	...	10	183		s	25.8-25.9
July 12	2	0	...	...	...	0	*	3	...	21	...	156	131	...	...	48	...	...	...	15	...	...	...	1	...	...	9	375	nm	s	25.0-26.0
	3	0	...	...	...	0	...	6	...	61	...	91	80	...	...	21	...	...	...	14	...	...	...	3	...	...	9	276		s	25.0-26.0
July 13	2	...	0	...	...	...	...	0	1 *	16	...	...	...	24	...	...	...	...	...	11	...	...	...	...	1	...	7	53		c	26.1-26.2
	3	...	0	...	...	...	...	16	135	...	151	...	...	75	...	...	...	...	...	32	...	...	...	...	5	...	7	414		c	26.1-26.2
July 14	3	...	0	...	...	...	...	5	...	21	...	19 *	...	19	...	...	...	3	...	...	...	...	...	0	...	...	7	67		r	26.3-26.5
	4	...	0	...	...	...	...	1	...	1	...	62	...	54	...	...	16	...	...	...	...	...	...	4	...	...	7	138		c	26.3-26.5
July 15	4	...	...	...	0	...	...	0	4	43	80	76	25	*	...	14	...	3	...	...	...	...	...	...	...	...	9	245		s	26.2-26.8
July 16	4	...	...	...	...	...	...	...	14	54	89	56	17	7	...	*	...	0	...	...	...	...	...	...	...	...	7	237		s	26.6-26.8
July 17	4	...	...	...	...	...	...	...	52	132	107	38	...	13	...	2	...	*	...	...	...	...	...	...	...	...	6	344		c	26.6-26.8
July 18	4	...	...	...	...	...	...	1	...	1	...	11	...	...	4	...	...	...	...	*	...	...	...	...	...	...	4	17		c	26.0-26.5
July 19	5	...	...	...	...	1	...	19	...	52	...	...	...	42	...	...	...	36	...	...	...	7 *	...	...	...	...	6	157		c	26.2-26.6
July 20	5	...	...	...	0	...	...	9	...	29	...	59	...	...	...	37	...	...	...	12	...	...	...	...	...	...	6	146	fq	r	26.2-26.5
July 21	5	...	...	...	**	...	...	...	...	67	...	...	...	...	...	...	...	59	...	...	...	18	...	...	...	...	3	144		s	26.2-27.0
July 22	5	...	...	...	...	...	...	13	**	30	...	9	...	...	...	...	...	0	...	...	...	...	...	...	...	...	4	52		s	26.9-27.1
July 23	5	...	...	...	...	...	...	...	1	...	...	2	...	...	...	**	...	0	...	...	...	...	...	...	...	...	3	3		s	27.0-27.6

*: high tide, **: low tide, ...: no collection, Nc: number of collections in one day, Mc: total number of medusae collected in one day, lq: last quarter, nm: new moon, fq: first quarter, c: cloudy, s: sunny, r: rainy,
T: range of water temperature.

maximal number of medusae released per colony in one day was 696 (July 11 for colony 2) (Table 2). In three colonies observations were made every 15 or 30 minutes between 18:45 and 20:00 hr on July, 11, 12, 15, and 16 (Table 2) to detect differences in the release peak (18:45 - 20:00 hr). The number of collected medusae was greatest between 19:00 and 19:30 hr (p <0.01), the period just before and immediately after sunset (19:10 hr).

(2) Infulence of light as a factor synchronizing medusa release

Changes in moonlight, tidal cycles and weather conditions did not correlate with release of medusae (Table 2). The release was very strongly correlated with changes in light intensity that occur during sunset and perhaps act as a trigger for synchronous release of medusae and also for spawning of gametes.

During artificial darkness experiments, a total of 1417 medusae was collected. Artificial darkness preceding true sunset (19:10 hr) caused release of medusae two hours earlier than under ordinary conditions (e.g. 12.3% vs 0.2% released before 17:30), which constituted a significant difference in timing (p < 0.01) (Table 3, Ad).

(3) Darkness may work as a stop signal for medusa release

Under natural conditions, no medusae were collected after 23:00 hr and only 14.6% of medusae were released between 20:15 and 23:00 hr (Table 2, Nc). Timing was changed by prolonged light illumination. In the delayed sunset experiment a total of 1734 medusae was released and the number of medusae liberated between 20:15 and 23:00 hr was significantly different from that observed under normal conditions (p < 0.01) (Table 3, Ads). Even for the number of medusae collected between 21:15 and 23:00 hr, differences were clear (17.2% vs 5.6% under natural conditions, p < 0.01).

In colonies 6, 7, and 8 (Table 1), collections were made every 15-30 minutes between 18:45-20:00 hr on July 26, 27, and 30. A total of 798 medusae was collected. The release peak occurred later than that under natural conditions (after 19:30, 49.6% vs 27.7%, p < 0.01).

These results suggest there is a light-inhibitory mechanism involved in medusa release, because medusae usually were not liberated after 1 to 2 hr of darkness.

(4) Release of gametes and swimming activity of medusae

Most of medusae collected before and during the release peak (see above) showed active swimming movements, but only for a few minutes. Medusae collected after sunset were found on the bottom of the petri dish and showed only weak pulsations or none at all (Table 4).

Male medusae and femele medusae had similar bell heights (0.99 mm on average, SD= 0.12, N= 35 males; and 1.03 mm on average, SD= 0.11, N= 39 females) but the diameter of males was smaller than that of females (0.61 mm on average, SD= 0.07, N= 35 vs 0.72 mm on average; SD=0.12, N=39; p< 0.1). This difference was due to the presence of large spherical eggs in females (0.5 mm in

Table 3. Temporal changes in number (N) and frequency (%) of medusae released by *Halocordyle disticha* (sunset was at 19:10 hr).

	0:00-17:30	17:30-18:30	18:45-20:00	20:15-21:00	21:15-23:00
Exper. cond.*	N (%)	N (%)	N (%)	N (%)	N (%)
Nc	8 (0.2)	97 (2.6)	3062 (82.5)	335 (9.0)	208 (5.6)
Ad	174 (12.3)	350 (24.7)	746 (50.6)	104 (7.3)	43 (3.0)
Ads	0	25 (1.4)	1102 (63.6)	308 (17.8)	299 (17.2)

* Nc: natural conditions. Ad: artificial darkness. Ads: artificially delayed sunset.

Table 4. Temporal changes in number (N) and frequency (%) of swimming activity of medusae released by *Halocordyle disticha* (sunset was at 19:10 hr).

	0:00-17:30	17:30-18:30	18:45-20:00	20:15-21:00	21:15-23:00
Activity*	N (%)	N (%)	N (%)	N (%)	N (%)
S	8 (100)	90 (98.2)	1775 (58.0)	24 (7.2)	0
P	0	6 (6.2)	799 (26.1)	106 (31.6)	27 (13.0)
N	0	1 (1.0)	488 (15.9)	205 (61.2)	181 (87.0)

* S: swimming well. P: not swimming but pulsating on the bottom of the petri dish.
N: neither swimming nor pulasating.

diameter, with some oval ones up to 0.7 mm), which caused the bell margin to expand. There were up to 4 eggs in a medusa.

Many medusae spawned gametes as a result of strong bell pulsations. However, most had shed their sexual products prior to collection, and many eggs were found in the sea water. It is surmised that the spawning is almost simultaneous with medusa release. Brinckman-Voss (1970) noted that gametes in the Naples population were shed from some gonophores that were still attached to the hydroids. Eumedusoids of *H. disticha* thus act as simple gamete carriers and their planktonic life is very ephemeral.

(5) Ecological implications of simultaneous release of mature medusae

Successful synchronization of gamete release maximizes the probability of achieving fertilization. In dioecious *H. disticha*, male and female colonies may live at some distance from each other, and the medusae are so ephemeral that simultaneous release and spawning increase the chances of fertilization.

Light is used by many hydrozoans as a cue to synchronize spawning. The accumulation of light-sensitive substances and the ratio of Ca^{++} and Mg^{++} ions play a very important role in the process of discharge of gametes or induction of maturation of germinal cells (Ballard, 1942; Yoshida, 1954; Yoshida *et al.*, 1980).

Kubota (1996) suggested a photosensitive mechanism in the release of medusoids of *Eugymnanthea japonica* Kubota, 1979, where the stimulus or the trigger of the release is a fall in light intensity. Spawning occurs around sunset both in species without medusa stages, such as *Clava* sp., and *Hydractinia equina* (Fleming, 1828) (Ballard, 1942), as well as in species with ephemeral medusae such as *Amphisbetia operculata* (L.) (Teissier, 1922), *Halocordyle disticha* (Ballard, 1942; Brinckmann-Voss, 1970), *Hydractinia epiconcha* (Yoshida, 1954), and *Eugymnanthea japonica* (Kubota, 1996, 1997).

Another variation in the life cycle comprises two long stages with morphological and ecological differences, namely a benthic modular polyp and an individual planktonic medusa. Medusae are released with undeveloped or immature gonads and live for a prolonged period (days/weeks/months) in the plankton. Spawning in hydromedusae is also usually controlled by photic stimuli; circadian periodicities have been reported in some species of hydromedusa such as *Phialidium* (=*Clytia*) *gregarium* (Rosen-Runge, 1962); *P.* (=*C.*) *hemisphericum* (Honegger *et al.*, 1980), *Spirocodon saltatrix* (Tilecius) (Yoshida *et al.*, 1980) , and *Eutima japonica* Uchida, 1925 (Kubota, 1997).

Two kinds of timing mechanism need to be present in such metagenetic life cycles: for synchronous release of immature medusa, and for synchronous spawning of gametes. In spite of evolutionary outcomes resulting in changes in timing (heterochrony) (Boero *et al.*, 1997), there have been no studies of possible linkage between the timing of spawning and medusa release, and this subject deserves futher study.

Aknowledgements

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